EFFICACY OF FOLIC ACID PROPHYLAXIS FOR THE PREVENTION OF NEURAL TUBE DEFECTS

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Thirty years ago, researchers suggested that maternal intake of certain vitamins during pregnancy affected the incidence of serious birth defects. Since then, two randomized controlled trials and several observational studies have proven that if women take folic acid during the periconceptional period, they can lower their risk of having children with neural tube defects (NTDs), serious birth defects of the spine and brain. In 1992, the U.S. Public Health Service recommended that all women capable of becoming pregnant take 0.4 mg of folic acid daily. Translating this recommendation into practice, however, presents a major public health challenge. In 1996, the U.S. Food and Drug Administration ruled that "enriched" cereal grain products must be fortified with folic acid, the first time food has been fortified for the prevention of birth defects. However, because the level chosen for folic acid fortification will not provide all women the optimal protection against the occurrence of NTDs, efforts to increase reproductive-age women's consumption of folic acid-containing vitamins and folate-rich foods are underway. The mechanism underlying folic acid's efficacy in preventing NTDs is unknown. It may work by correcting a deficiency or by overcoming an inherited disorder of folate metabolism. The role of genetics and agents such as vitamin B_{12}, methionine, and homocysteine in NTD prevention, and the relationship of these factors with folic acid, are under investigation. Although the mechanism for folic acid's protective effect is unknown, it is clear that a significant proportion of NTDs can be prevented and that prevention efforts should not await the elucidation of specific mechanisms. © 1998 Wiley-Liss, Inc.
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Because the etiologies for most birth defects are unknown, the finding that maternal consumption of folic acid can prevent some neural tube defects (NTDs) is an exciting chapter in birth defect prevention. There has been a proliferation of articles on this subject in recent years. A Medline search on the subject of "neural tube defect" combined with "folic acid" yielded 27 articles published from 1981–1986, 53 from 1987–1992, and 245 from 1993–January 1998. Research results supporting the finding of a protective effect of maternal use of folic acid on the occurrence of many NTDs are compelling. Not since rubella vaccine became available 30 years ago for the prevention of congenital rubella syndrome has there been such an opportunity for the primary prevention of birth defects. This article addresses the *efficacy* of folic acid prophylaxis in preventing neural tube defects, and discusses the challenge of assessing the *effectiveness* of interventions.

FOLIC ACID

Wills and Mehta [1930] discovered a factor that cured the nutritional deficiency anemia of pregnancy among women in India. Mitchell et al. [1941] later isolated this "Wills' factor" from spinach leaves and called it "folic acid" (*folium* is Latin for "leaf"). Folic acid is a water-soluble B vitamin that has had several aliases (e.g., vitamin B_c, vitamin M, *Lactobacillus casei* factor) [Herbert and Colman, 1988]. Wald [1996] suggested that it be relabeled as "vitamin B4" to avoid the negative perception by some women of consuming an "acid" and to communicate the necessity of taking the vitamin *before* pregnancy.

The term "folate" refers to a class of food compounds that have the biologic activity of folic acid. Folate is an essential nutrient; it is synthesized by microorganisms and higher plants, but not by mammals. Although there are folate-producing bacteria in the human gut, they do not appear to contribute significant amounts of folate, and man is wholly dependent upon dietary sources [McNulty, 1995]. Folates are widely distributed in foods. Concentrated food sources include green leafy vegetables, grains, legumes, certain fruits, and liver. Folates in foods exist primarily as polyglutamates. Synthetic folic acid is in the monoglutamate form. Heat, ultraviolet light, and air inactivate food folate [Herbert, 1987]. Therefore, food processing, preparation, and cooking can reduce the amount of food folate ingested by an estimated 50–95% [Herbert and Colman, 1988]. *Bioavailability*, the extent to which folates are available for use at the cellular level, is a very complex and multifaceted phenomenon that is influenced by many factors [Gregory, 1995]. An estimated one half to two thirds of food folate is bioavailable [Herbert, 1987], although there is wide variability in the percentage of folate that is bioavailable in various foods, rendering estimates of bioavailable folate difficult and imprecise. The recent report of the Institute of Medicine (IOM), National Academy of Sciences [1998] estimated the bioavailability of synthetic folic acid to be about twice that of food folate. Studies have consistently demonstrated greater bioavailability of monoglutamate over polyglutamate forms of folate [McNulty, 1995], which is of note because the synthetic form of folic acid used in

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cereal grain fortification and vitamin supplements contains only the monoglutamate form. The fact that fortified food and supplements can deliver folic acid in a more bioavailable form than food folate has implications for interventions designed to prevent NTDs.

FOLIC ACID AND NTDs

The most interesting work relative to NTD etiology and prevention in the last few years has focused on the effect of nutrition. The increased prevalence of NTDs in lower socioeconomic groups, the decreasing NTD rates, and the effects of seasonality on NTD rates were all consistent with a dietary etiology for NTDs [Elwood et al., 1992]. Vitamins, specifically folic acid, were thought to be involved because of their role in human growth and because birth defects occurred with maternal use of agents which inhibited the vitamin's action (e.g., aminopterin, a folic acid antagonist, when used as an abortifacient, induced fetal NTDs) [Thiersch, 1952].

REVIEW OF RESEARCH

Early Studies

A link between folate deficiency and human fetal malformations (although not NTD malformations) was first reported by Hibbard [1964]. Smithells et al. [1976] found that women with NTD-affected pregnancies had lower levels of red blood cell folate and other vitamins than those with unaffected pregnancies. A dietary study in South Wales suggested that women on adequate diets had a lower recurrence rate for NTD pregnancies than women on poor diets [Laurence et al., 1981].

Intervention Studies

Following their dietary studies, Smithells et al. [1976] and Laurence et al. [1981] conducted intervention studies. In the discussion of these studies, we use "recurrence" to describe the NTD-affected pregnancy of a woman who has had a previous affected pregnancy and "occurrence" to describe the NTD-affected pregnancy of a woman without such a history. Approximately 95% of all NTD-affected births are to women with no previous history of NTD-affected pregnancy. The recurrence prevention trial of Smithells et al. [1980] used a multivitamin supplement delivering a daily dosage of 0.36 mg of folic acid. The comparison group consisted of women who were already pregnant or had refused participation, because permission was not granted to perform a randomized

trial [Elwood et al., 1992]. This study showed a 91% reduction in the recurrence in the Yorkshire region of the United Kingdom [Smithells et al., 1989] and an 83% reduction in Northern Ireland [Nevin and Seller, 1990]. Criticism of the study is reviewed in detail elsewhere [Elwood et al., 1992], and centered around the nonrandomization design; concern that the findings could be due to selection bias (participants who took folic acid-containing vitamins were self-selected and had higher socioeconomic status); that the study was not double-blind; and that there was no placebo component.

The fact that fortified food and supplements can deliver folic acid in a more bioavailable form than food folate has implications for interventions designed to prevent NTDs.

Laurence et al. [1981] also conducted a small *randomized* recurrence prevention trial, using 4 mg of folic acid or a placebo, and demonstrated a nonsignificant 58% reduction in risk for an NTD-affected pregnancy among folic acid users. A nonrandomized recurrence prevention trial in Cuba found no affected pregnancies among 101 women who took 5 mg of folic acid supplements during early pregnancy compared with four NTD-affected pregnancies among 118 unsupplemented women [Vergel et al., 1990].

Results from the observational and intervention studies are summarized in Figure 1 and Tables 1 and 2. Two randomized controlled trials deserve special comment. The large trial of the United Kingdom's Medical Research Council (MRC) Vitamin Study Research Group [1991], a recurrence prevention trial, was conducted at 33 sites in seven countries, although most participants came from Great Britain and Hungary. In all, 1,817 women at risk of having an NTD-affected pregnancy because of a previous affected pregnancy were randomized to one of four vitamin use groups: 1) 4 mg folic acid daily, 2) other vitamins not including folic acid, 3) both folic acid and other vitamins, or 4) no vitamin supplements. High-dose

folic acid (4 mg) was used to avoid the attribution of a possible negative result to inadequate dosage. Among 1,195 pregnancies in which the outcome was known, women who had received folic acid (alone or with other vitamins) had an NTD-affected pregnancy rate of 1%, and women who had not received folic acid had a rate of 3.5%, a significant 72% risk reduction among folic acid users. The other vitamin group showed no significant risk reduction (relative risk, 0.80; 95% confidence interval (CI), 0.32–1.72). Thus the MRC trial provided the first strong evidence that the relevant nutritional agent was folic acid. Following the release of these findings, the U.S. Centers for Disease Control and Prevention (CDC) issued a recommendation stating that women who had a previous NTD-affected pregnancy should take 4 mg of folic acid daily when planning a subsequent pregnancy [Centers for Disease Control and Prevention, 1991].

The results of the recurrence prevention trials were not considered to be appropriate enough to make a policy recommendation for the use of folic acid in occurrence prevention. Soon after, however, in 1992, the results of the Hungarian occurrence prevention randomized trial provided strong evidence for efficacy in women who had *not* had a previous NTD-affected pregnancy. Of 2,052 offspring of women who took a trace element supplement without folic acid, six had NTDs, as opposed to none of the 2,104 offspring of women who took a daily multivitamin containing 0.8 mg of folic acid [Czeizel and Dudas, 1992]. With the news of these results and the results from several observational studies, the evidence for folic acid's preventive effect among women with no history of an NTD-affected pregnancy was convincing. Thus, in September 1992, the U.S. Public Health Service (PHS) recommended that *all* women capable of becoming pregnant consume 0.4 mg (400 μg) of folic acid per day [Centers for Disease Control and Prevention, 1992]. The recommendation was made for all women because at least half of U.S. pregnancies are unplanned or mistimed and because these birth defects occur very early in pregnancy (3–4 weeks after conception), before many women are aware that they are pregnant.

Observational Studies

Although observational studies do not provide scientific proof as strong as that of randomized trials, they do provide useful evidence, especially if replicated in various settings. All but one observational

Table 1. Intervention Studies of Folic Acid Consumption and the Occurrence of NTDs

Study	Design	Subjects	Exposure	Results	Comments
Smithells et al., 1980, 1983	Nonrandomized controlled multicenter trial in UK	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 0.36 mg folic acid + multivitamins daily. Unsupplemented mothers took nothing.	Women were given 0.36 mg folic acid + multivitamins or reported no use from 1 month before conception through the 1st trimester.	3 NTD-pregnancies among 454 supplemented women. 24 NTD-pregnancies among 519 unsupplemented women. Relative risk = 0.14, $P < 0.05$.	86% reduction in risk.
Laurence et al., 1981	Randomized controlled trial in Wales	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 4 mg folic acid daily. Unsupplemented mothers took a placebo.	Supplemented women were given 4 mg folic acid daily at least 1 month before conception through the 1st trimester.	2 NTD-pregnancies among 60 supplemented mothers. 4 NTD-pregnancies among 51 unsupplemented mothers. Relative risk = 0.40, not statistically significant.	60% reduction in risk.
MRC study, 1991 (UK)	Randomized controlled multicenter trial in UK and Hungary	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 4 mg folic acid daily. Unsupplemented mothers took a placebo.	Women were given 4 mg folic acid or placebo daily at least 1 month before conception through the 1st trimester.	6 NTD-pregnancies among 593 supplemented women. 21 NTD-pregnancies among 602 unsupplemented women. Relative risk = 0.28, $P < 0.05$.	72% reduction in risk.
Vergel et al., 1990	Nonrandomized controlled trial in Cuba	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 5 mg folic acid daily. Unsupplemented mothers took nothing.	Women were given 5 mg folic acid or reported no use from 1 month before conception through the 1st trimester.	0 NTD-pregnancies among 81 supplemented women. 4 NTD-pregnancies among 114 untreated women. Indeterminate protective effect, not statistically significant.	Complete protective effect.
Czeizel and Dudas, 1992	Randomized controlled trial	Pregnant women without prior NTD-affected pregnancy: Supplemented mothers took 0.8 mg folic acid + multivitamins daily. Unsupplemented mothers took trace elements.	Women were given 0.8 mg folic acid + multivitamins or placebo daily from 1 month before conception through the 1st trimester.	0 NTD-pregnancies among 2,104 supplemented women. 6 NTD-pregnancies among 2,052 unsupplemented women. Indeterminate protective effect, $P < 0.05$.	Complete protective effect.

ticipants knew the outcome of their pregnancy.

Periconceptional folic acid use does not protect against the occurrence of all NTDs. However, based on the risk reduction observed in several studies, estimates are that at least 50% of NTDs could be prevented by the use of folic acid [Centers for Disease Control and Prevention, 1992].

Efficacy Issues

Some issues relative to folic acid efficacy deserve discussion. Some studies have not shown significant differences between serum or red-cell folate levels in women with NTD-affected pregnancies and those with unaffected pregnancies, and folate levels are often within "normal" ranges in mothers with affected infants [Scott et al., 1994]. These findings could be due to the size or characteristics of the populations studied, the time at which folate levels were determined, or a narrow range of folate values. Another possibility is that women with NTD-affected pregnancies have an impairment in the metabolism of folic acid rather than inadequate intake per se. However, a

study in Dublin, Ireland [Daly et al., 1995], found that a woman's risk of having an NTD-affected pregnancy was inversely associated with her early-pregnancy red blood cell (RBC) folate levels in a dose-response relationship. As RBC folate levels increased, the NTD rate decreased from 6.6 per 1,000 births to 0.8 per 1,000 births. Furthermore, the rate decreased as RBC folate levels increased well past what is considered clinically "normal." This study is without some of the potential bias of other blood studies because samples were obtained from pregnant women early in pregnancy before the pregnancy outcome was known and closer to the time of neural tube formation.

Dosage

The minimum daily fully-effective dosage of folic acid needed for the prevention of NTDs is unknown. For recurrence prevention, the Medical Research Council Vitamin Study Research Group [1991] and Laurence et al. [1981] used 4 mg, and the Cuban study [Vergel et al., 1990] used 5 mg, yet Smithells' [1983] nonrandomized study found a protective effect using only 0.36 mg

daily. For occurrence prevention, the PHS recommendation of 0.4 mg (400 μg) was based on the protective effects found in Smithells' study and in the various observational studies [Mulinare et al., 1988; Milunsky et al., 1989; Werler et al., 1993]. Results from the large community intervention in China will be important in that this is the first active intervention to use the 0.4 mg supplement alone for NTD occurrence prevention. The minimum effective dose may be less than 4 mg for recurrence prevention and less than 0.4 mg for occurrence prevention. However, determining the effectiveness of lower dosages may be difficult because of the size and cost of studies necessary to assess the relative efficacy of various dosages.

Special Populations

Some studies have suggested that folic acid may be less protective in certain populations. Shaw et al. [1995a] found that the NTD risk reduction associated with folic acid use was less marked for Hispanics than for non-Hispanic whites or blacks. However, the risk varied within the group; the odds ratio for folic acid-containing vitamin users compared
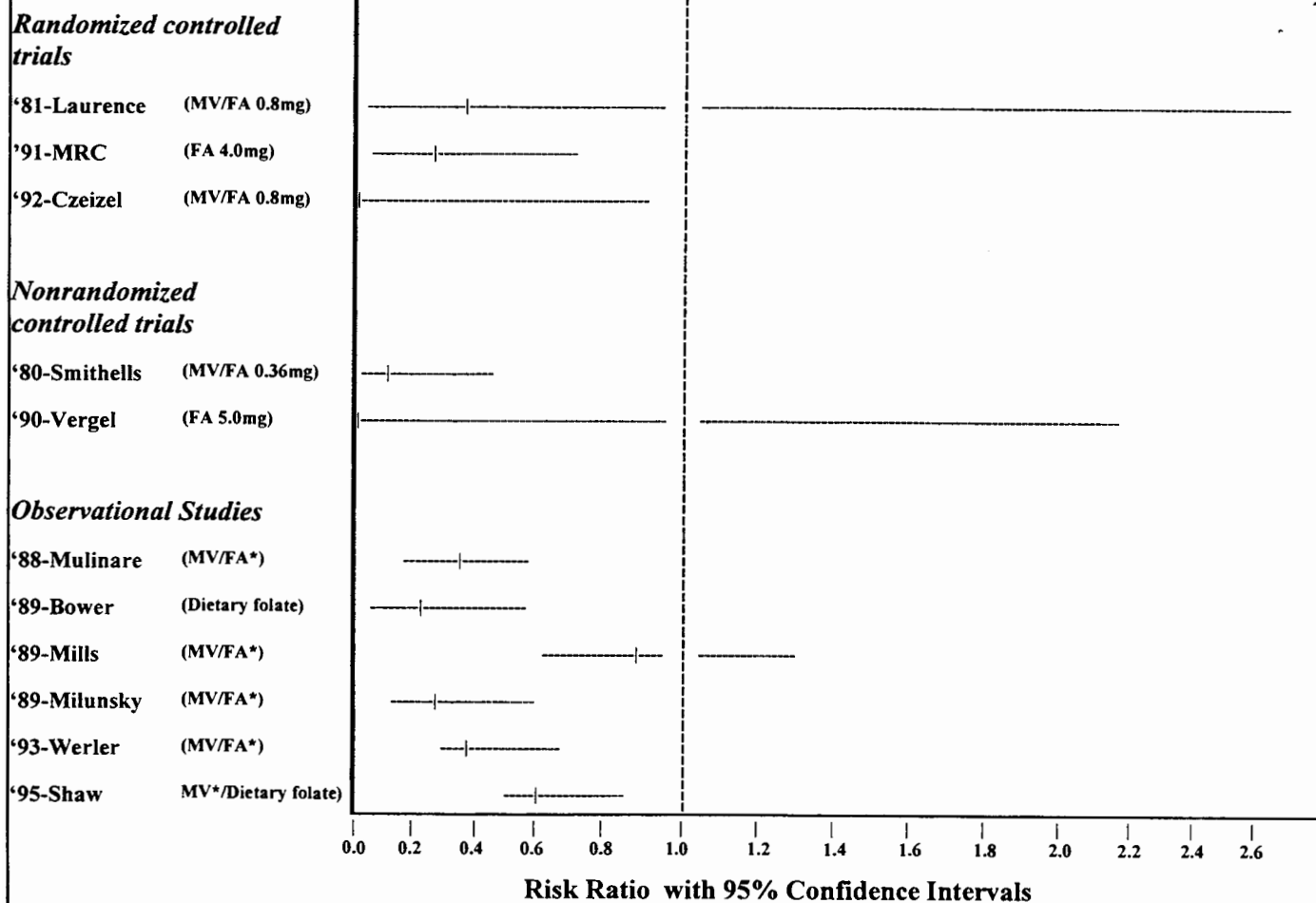
Fig. 1. Risk ratios for neural tube defect-affected pregnancies, comparing women who used multivitamin (MV) or folic acid (FA) supplements with those that did not, by study type, 1980–1995. *Designated observational studies used folic acid (0.1–1.0 mg).

study supported the findings of the intervention studies that folic acid has an effect against NTDs. A study in Atlanta, Georgia, was the first U.S. population-based study to show that women who used multivitamin supplements periconceptionally had a lower risk for NTD-affected pregnancies than non-supplement users [Mulinare et al., 1988]. In an Australian study, Bower and Stanley [1989] found a reduced risk for NTD-affected pregnancies among women with higher early pregnancy folate intake (from supplements and foods). Later, a New England study reported a protective effect of periconceptional use of vitamins with folic acid, as well as decreasing NTD risks associated with increasing dietary folate intake [Werler et al., 1993].

A case-control study of women in Illinois and California did not find a protective effect for NTDs among women who used vitamin supplements [Mills et al., 1989]. The reason for the null effect in Mills et al. [1989] is unknown. One proposed explanation was that possible underascertainment of cases and the definition of vitamin exposure could have resulted in misclassification [Milunsky et al., 1989, 1990; Shaw et al., 1995a]. An alternative explanation is that the effect of vitamin supplementation is greater in areas with higher NTD prevalence than in low-prevalence areas such as California and Illinois [Nevin and Seller, 1990]. However, a subsequent California study found that the risk of an NTD-affected pregnancy was 35% lower among women who took vitamins containing folic acid [Shaw et al., 1995a], which is inconsistent with Mills et al. [1989] and the low-prevalence hypothesis. A community intervention with folic acid in the People's Republic of China cosponsored by the CDC and Beijing Medical University will help clarify whether folic acid's protective effect varies by NTD prevalence, because it is being conducted in both high-prevalence (northern provinces) and low-prevalence (southern provinces) areas.

A theoretical concern in retrospective studies is that differential recall may bias the results (i.e., mothers of affected infants may be more likely to recall "exposures" such as vitamin use during pregnancy than mothers of unaffected infants). One way to address this is to evaluate the effect of maternal folic acid consumption in a control group of infants with birth defects other than NTDs, although this approach is not without problems. The case-control studies of Mulinare et al. [1988], Bower and Stanley [1989], and Werler et al. [1993] demonstrated that folic acid had a protective effect whether the comparison group was healthy infants or those with other birth defects. Furthermore, another study [Milunsky et al., 1989] reported that use of multivitamins containing folic acid was protective in a cohort study of 22,776 women, 49 of whom had an NTD-affected pregnancy. Recall bias relative to folic acid consumption was not a concern in this prospective cohort study because the ascertainment of vitamin consumption occurred during pregnancy (during prenatal screening for maternal serum alpha fetoprotein screening), before par-

Table 2. Observational Studies of Folic Acid Consumption and the Occurrence of NTDs

Study	Design	Subjects	Exposure	Results	Comments
Mulinare et al., 1988	Case/control in metropolitan Atlanta	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin supplement containing 0–0.8 mg of folic acid at least 1 month before conception through the 1st trimester.	24 supplemented and 157 unsupplemented NTD case women. 405 supplemented and 1,075 unsupplemented women controls. Odds ratio = 0.40, $P < 0.05$.	60% reduction in risk.
Bower and Stanley, 1989	Case/control in Western Australia	Spina bifida case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Dietary folate and multivitamin supplement at least 1 month before conception through the 1st trimester.	77 NTD cases; 154 control mothers in study. The highest folate quartile was compared with the lowest. An increasing protective effect was observed from the lowest to the highest quartile. Odds ratio = 0.25, $P < 0.05$.	75% reduction in risk.
Mills et al., 1989	Case/control in California and Illinois	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin + folate supplement containing up to 0.8 mg folic acid + diet at least 1 month before conception through the 1st trimester.	89 supplemented and 214 unsupplemented NTD case women. 90 supplemented and 196 unsupplemented women controls. Odds ratio = 0.91, not statistically significant.	No protective effect.
Milunsky et al., 1989	Prospective cohort in New England	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin + folate supplement containing 0.1–1.0 mg folic acid + diet at least 1 month before conception through the 1st trimester.	10 NTD-pregnancies among 10,713 women who took multivitamin + folate. 39 NTD-pregnancies among 11,944 women who took multivitamins without folate. Relative risk = 0.28, $P < 0.05$.	72% reduction in risk.
Werler et al., 1993	Case/control in Boston, Philadelphia, and Toronto	NTD case babies and abnormal control babies (babies with other birth defects). Pregnant women without a prior NTD-affected pregnancy.	Multivitamin + folate supplement containing 0.4–1.0 mg folic acid + diet at least 1 month before conception through the 1st trimester.	24 supplemented and 157 unsupplemented NTD case-women. 405 supplemented and 1,075 unsupplemented women controls. Odds ratio = 0.40, $P < 0.05$.	50% reduction in risk.
Shaw et al., 1995	Case/control in California	NTD-affected pregnancies and babies, and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Dietary folate and multivitamin supplement 3 months before conception, and/or 3 months after conception	410 supplemented and 482 unsupplemented NTD case women. 207 supplemented and 149 unsupplemented women controls. Odds ratio = 0.61, $P < 0.001$.	39% reduction in risk.

to nonusers was 0.48 for first-generation Hispanics, but 2.1 for second-generation or more women, and neither was statistically significant. The reason for the less marked risk reduction among first-generation Hispanics is unknown. More research is needed to see if this finding can be replicated and, if so, to explore potential reasons for it. A case-control study being performed among Hispanic women along the Texas-Mexico border may provide information in the near future (Mulinare, CDC, personal communication). By itself, the evidence from this one study is not strong enough to suggest that the recommendation for folic acid consumption be modified for Hispanic women.

Maternal obesity has been identified in several studies as a risk factor for NTDs [Waller et al., 1994; Shaw et al., 1996; Werler et al., 1996; Watkins et al., 1996]. The mechanism for this is unknown and may or may not be mediated by folic acid. Werler et al. [1996] found that intakes of folate of 0.4 mg or more reduced the risk for NTD-affected pregnancies among women weighing less than 70 kg but did not significantly reduce risk among heavier women. The other studies were unable to assess this interaction between obesity and folate due to lack of dietary data or sparse numbers. More study is needed to clarify the relationship and possible interactions between obesity, NTDs, and folate.

Other Dietary Factors

Folic acid is involved in complex biochemical pathways that also include methionine, an essential amino acid, vitamin B_{12}, and homocysteine. The relationships between these agents and NTDs have been the subject of numerous studies.

There is evidence for a link between vitamin B_{12} and NTD prevention, although it is not as strong as for folic acid. Schorah et al. [1980] reported lower serum B_{12} levels in mothers of infants with anencephaly than in control mothers. Perhaps the strongest evidence for an independent role for vitamin B_{12} comes from a study in Ireland [Kirke et al., 1993], which found lower early-pregnancy folate and B_{12} levels in mothers of

NTD-affected infants than in mothers of unaffected infants. Lower plasma B_{12} and plasma folate levels were both independent risk factors for having an NTD-affected pregnancy. The authors hypothesized that because the methionine synthase enzyme is independently influenced by both folate and B_{12}, this enzyme is somehow involved in NTD etiology. The authors called for more studies of the independent protective effect of vitamin B_{12} and suggested that consideration be given to including B_{12} in food fortification.

The link between NTD risk and maternal homocysteine metabolism has also been studied [Steegers-Theunissen et al., 1994, 1995; Mills et al., 1995]. In Steegers-Theunissen et al. [1994], almost a third of mothers of NTD-affected infants had methionine intolerance (and elevated homocysteine levels after oral methionine challenge), suggesting a possible metabolic block in the conversion of homocysteine to methionine. Folate may have been involved, because inadequate folate intake could result in insufficient methyltetrahydrofolate for conversion of homocysteine to methionine.

A link between methionine in NTD formation has been suggested by animal studies [Coehlo et al., 1989; Coehlo and Klein, 1990; Eissein and Wannberg, 1993; Nosel and Klein, 1992], and recently a 30–40% NTD risk reduction was reported among women whose average reported methionine intake was above the lowest quartile [Shaw et al., 1997]. This risk reduction persisted irrespective of folate intake. These findings need replication, and there were limitations (e.g., methionine correlated with several other nutrients; difficulty in accurately estimating methionine intake). Nonetheless, the findings are intriguing. In summary, the relationship between NTDs, vitamin B_{12}, homocysteine, methionine, and folic acid needs further elucidation.

MECHANISM

The underlying biologic mechanism by which periconceptional folic acid use protects against NTDs is unknown. Folic acid is involved in DNA synthesis and is therefore essential to the rapid cell division that occurs in early fetal development. It also plays a role in DNA methylation and thus in gene regulation. It is not clear whether prophylaxis works by correcting a folate deficiency in some women or by overriding an inherited disorder of folate metabolism. There is much interest in the role of mutations in the genes that code for enzymes involved

in folic acid metabolism [Hall, this volume]. However, the existence of a genetically mediated metabolic abnormality that contributes to folate deficiency would not discount the role of nutritional factors. Extrinsic folic acid may very well increase tissue folate levels enough to override a failure in folate metabolism.

Although the mechanism for folic acid's protective effect is unknown, it is clear that a significant proportion of NTDs can be prevented and that prevention efforts should not await the elucidation of specific mechanisms. The challenge becomes how to translate science into public health practice.

Hook and Czeizel [1997] used data from two trials [Medical Research Council, 1991; Czeizel and Dudas, 1992] to report a 16% higher miscarriage rate among women who used periconceptional folic acid. The authors proposed two possible hypotheses: 1) that folic acid may lower NTD birth prevalence through the mechanism of *terathanasia*, a term used to describe a process which selectively induces the miscarriage of a fetus with a neural tube defect, and alternatively, 2) that folic acid may sustain NTD-affected pregnancies that would otherwise have been spontaneously aborted so early that the miscarriage would have escaped detection. Critics of this analysis refute the report of a higher miscarriage rate among the folic acid-supplemented group and the practice of reporting spurious associations from post hoc analyses, and cite the biologic implausibility of the terathanasia explanation, especially in view of the finding of increased fertility and healthy live-born rates in the folic acid group [Wald and Hackshaw, 1997; Burn and Fisk, 1997; Hall, 1997; Schorah et al., 1997]. The finding of Hook and Czeizel [1997] that folic acid may be associated with the spontaneous abortion of defective conceptuses merits further study, as do other theories concerning the mechanism by which folic acid prevents NTDs.

APPROACHES TO INCREASII FOLIC ACID CONSUMPTION

Although we do not understa the mechanism underlying folic acic protective effect against NTDs, it is cle. that a significant proportion of NTDs ca be prevented. The challenge become how to translate science into public health policy and "real world" practice. The PHS recommendation suggested three approaches for increasing folic acid consumption: 1) promote increased consumption of folate-rich foods, 2) promote the use of folic acid-containing vitamins, and 3) fortify the food supply with additional folic acid.

Several folate-rich foods (e.g., fruits, leafy green vegetables, grains) are considered "healthy" because they are rich in other beneficial nutrients, high in fiber, and low in fat. The promotion of these foods thus may have the collateral advantage of conferring other health benefits such as reduced risk for cardiovascular disease, obesity, and cancer. However, U.S. women consume an average of only 200 µg folate daily from food sources [Subar et al., 1989]. Given this level of consumption of dietary folate and the fact that dietary sources of folate do not appear to be as bioavailable as the synthetic folic acid form, women would have to significantly increase their consumption of folate-rich foods to reach the equivalent of 400 µg of folic acid daily. Such increases are unlikely; it appears that women can only marginally increase their blood folate levels through dietary sources [Cuskelly et al., 1996]. A major and sustained dietary behavior change by the majority of women would be necessary. Efforts to increase the consumption of fruits and vegetables have not been dramatically successful. Furthermore, although observational studies suggest that dietary sources of folate confer some protection against NTDs, the experimental studies, which offer the strongest epidemiologic evidence, used supplemental folic acid. The report of the Institute of Medicine, National Academy of Sciences [1998], recommends that women capable of becoming pregnant take 400 µg of synthetic folic acid per day (from fortified foods and/or a supplement) *in addition to food folate*.

Although vitamins containing folic acid are of proven efficacy in preventing NTDs, their consistent use would necessitate a sustained behavior change by most women. A 1997 telephone survey of 2,001 women aged 18–45 showed that 30% of women took a daily vitamin containing folic acid, up from 25% in 1995 [Centers for Disease Control and

Prevention, 1995b, 1997]. Another third of the women surveyed took a vitamin supplement less than daily or a vitamin or mineral supplement that did not contain folic acid [Centers for Disease Control and Prevention, 1998], suggesting that this population may be an appropriate target for educational interventions. Vitamin use is less common among younger women, less educated women, and those with lower incomes [Centers for Disease Control and Prevention, 1998], populations that are often harder to reach through health education efforts. The fact that 65% of women reported being willing to take a vitamin if their provider encouraged them to, whereas only 16% had actually been so encouraged (Katherine Lyon-Daniel, CDC, personal communication), suggests that health care professionals should also be a target for educational efforts.

Fortification of a food staple is an approach favored by many because it offers the advantage of wide population coverage at low cost without requiring women to change their behavior. Cost-effectiveness studies have demonstrated the economic benefit of food fortification, especially at higher fortification levels [Romano et al., 1995; Kelly et al., 1996]. Fortification is consistent with the public health tradition of treating the whole population for the benefit of a subset (as in adding iodine to table salt to prevent goiter). Cereal grains have been vehicles for food fortification for many years in the U.S. and several other countries.

In 1996, the U.S. Food and Drug Administration (FDA) ruled that effective January 1, 1998, all flour, corn meal, pasta, and rice labeled as "enriched" be fortified with folic acid [Food and Drug Administration, 1996]. This was the first new fortification since 1943 and the first time foods had been fortified to prevent birth defects. Cereal grains were chosen as the staple food for fortification because they are consumed daily in varying amounts by over 90% of women of childbearing age. There was debate over the ideal fortification level, and although some advocated a higher fortification level [Beresford, 1994; Oakley et al., 1995], the level of fortification chosen was 140 µg/100 g cereal grain product. Because food fortification would affect most of the population, there was concern that in some people the extra folic acid from fortified foods could prevent or resolve the anemia of vitamin B_{12} deficiency and delay the diagnosis, with the possible result of irreversible neuropathy. This seems unlikely and avoidable, how-

ever, because the diagnosis of B_{12} deficiency is now based on B_{12} measurement rather than on the presence of anemia. To address this concern, Herbert and Bigaouette [1997] suggested that cereal grain products also be fortified with vitamin B_{12} at a minimum of 25 µg B_{12} per 100 g cereal grain product or for each folic acid supplement. However, hard evidence about adverse effects associated with high doses of folic acid is lacking, and the concern about masking pernicious anemia is thought by many to be overstated and one that should not forestall the benefit associated with food fortification with folic acid [Bower and Wald, 1995; Dickinson, 1995; Oakley et al., 1996]. Furthermore, the report of the Institute of Medicine [1998] defined the adult tolerable upper limit for folate to be 1 mg of synthetic folic acid, in addition to the contribution from food folate.

... the U.S. Food and Drug Administration ruled that "enriched" cereal grain products must be fortified with folic acid, the first time food has been fortified for the prevention of birth defects.

The FDA also ruled that makers of foods supplying at least 10% (40 µg) of the recommended daily intake (R.D.I.) of folic acid per serving can advertise that the vitamin may reduce the risk of neural tube defects. It should be noted that although most breakfast cereals contain 25% (100 µg) of the R.D.I. of folic acid per serving, a few contain 100% (400 µg) of the R.D.I. per serving, the amount equivalent to that in most multivitamin supplements. Women can meet the recommended intake of 400 µg of folic acid by eating one serving of these "fully fortified" cereals (e.g., "Product 19," "Total"). However, these cereals have a small share of the total cereal market.

With the current level of folic acid fortification of cereal grains in the U.S., the CDC estimates that reproductive-age women's dietary intake of folic acid will increase by an estimated 100 µg/day, to about 300 µg/day, an amount likely to prevent some but not all of the estimated 2,000 cases of NTDs in the U.S. that

could be prevented by sufficient maternal folic acid consumption [Daly et al., 1995]. Although fortification will play an important part in the prevention of NTDs, it will not eliminate all of these "folic acid-preventable NTDs" [Brown et al., 1997; Daly et al., 1997].

Interventions and Their Effectiveness

An intervention study in Ireland provides some information about the effectiveness of these approaches. Women were randomly assigned to one of five groups: 1) those receiving a folic acid supplement (400 µg/day), 2) those receiving fortified foods (an additional 400 µg of folic acid/day), 3) those receiving natural foods containing dietary folate (an additional 400 µg/day), 4) those receiving dietary advice, and 5) a control group. Dietary *intake* of folic acid/folate increased significantly in all intervention groups. However, neither dietary advice nor folate-rich foods significantly increased women's *red cell folate levels*, whereas supplements and fortified foods significantly increased those levels by approximately 50% [Cuskelly et al., 1996].

Even with the increase in folic acid intake from the current level of fortification, most U.S. women will not consume 400 µg of folic acid daily. Therefore, activities to increase supplement use and consumption of folate-rich foods are underway. Their success will depend to a great extent on the successful implementation of educational and behavioral change interventions at the community level. There are no published population-based data that show a reduction in NTD rates as a result of public health efforts to promote folic acid consumption, although the results of the large community intervention in China using folic acid supplements are forthcoming.

Outcome studies that assess the impact of interventions on NTD rates are ongoing. However, they are resource-intensive and require ascertainment of prenatally diagnosed cases for accurate determination of NTD rates [Cragan et al., 1995]. Measurement of an intermediate outcome (e.g., knowledge about folic acid's importance and folic acid use) is less difficult. Most studies have demonstrated low baseline levels of knowledge about and periconceptional use of folic acid, and only modest increases since folic acid recommendations were made in the U.S. and other countries [Centers for Disease Control and Prevention, 1997; Clark and Fisk, 1994; Bower et al., 1997; Wild et al., 1997; Sayers et al., 1997], even among

women who had NTD-affected pregnancies [Centers for Disease Control and Prevention, 1995a; Forman et al., 1996]. A 1997 survey of 2,001 U.S. women aged 18–45 demonstrated that although 66% had heard of folic acid, only 11% knew that folic acid prevented birth defects, only 6% knew that it should be taken before pregnancy, and less than one third took a supplement containing folic acid daily, all modest increases from 2 years before [Centers for Disease Control and Prevention, 1995b,1997]. These findings demonstrate both the need for and the challenges inherent in disseminating knowledge to the public and in changing behavior.

Biological surveys of serum and blood folate levels have the potential to assess folic acid intake. Because blood folate levels correlate with folic acid consumption and appear to correlate with NTD rates [Daly et al., 1995], community surveys of blood folate levels could be used to estimate the proportion of a population that has adequate folate levels or to assess the effectiveness of an intervention with pre- and postintervention testing. In the U.S., the National Health and Nutrition Examination Surveys (NHANES) III and IV could be used to evaluate the impact of fortification and supplement use, and other biological surveys of specific populations in other settings are in the planning stages.

Future Directions

Obviously more research is needed in order to define the role of genetics; to ascertain the protective effect of folic acid in the prevention of other birth defects; to determine the underlying mechanism for that effect; to determine the relationships between folic acid, homocysteine, vitamin B_{12}, and methionine; and to ascertain other risk and protective factors for neural tube defects, both nutritional and otherwise. It will also be important to assess the extent to which fortification and educational and behavioral interventions increase folic acid consumption. In the U.S., the CDC has funded eight centers for birth defect research and prevention. All centers are conducting case-control studies of risk factors for NTDs and selected other birth defects, and several are conducting genetic studies and implementing NTD surveillance and prevention activities.

This review has focused on activities in the U.S. Other countries have instituted a variety of national policies and interventions [Cornell and Erickson, 1997]. Additional activities, planned

through the International Centre for Birth Defects (ICBD), involve birth defect registries that are part of an international network, the International Clearinghouse for Birth Defect Monitoring Systems. These international activities include studies to compare NTD prevalence before and after initiation of preventive strategies in several countries. These studies are valuable because they will allow researchers to compare the impact of interventions across countries with different NTD rates and different rates of folic acid use among reproductive-age women. Another ICBD activity planned for several countries is an anonymous newborn blood-spot survey of gene variants related to folate activity (Botto, ICBD, personal communication).

Some tantalizing, although not conclusive, evidence suggests that other types of birth defects may be prevented by periconceptional use of multivitamins or folic acid. These include orofacial clefts, some heart defects, limb-reduction defects, and urinary tract defects [Botto et al., 1996; Li et al., 1995; Shaw et al., 1995b; Tolarova and Harris, 1995; Czeizel, 1996]. A discussion of folic acid is not complete without mentioning its probable role in the prevention of cardiovascular disease, a popular topic in the scientific and lay press, but one beyond the scope of this review. It is clear that more research is needed, both into the etiology of NTDs and into how they can be prevented. However, lack of knowledge about underlying mechanisms that cause NTDs should not hinder prevention activities. Proof for the efficacy of folic acid prophylaxis is overwhelming, and prevention efforts should not await the exact elucidation of how folic acid works, especially since babies are born every day with spina bifida and anencephaly that could have been prevented. ∎

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